Enteral nutrients potentiate glucagon-like peptide-2 action and reduce dependence on parenteral nutrition in a rat model of human intestinal failure

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1Department of Surgery, University of Wisconsin-Madison, Madison, Wisconsin; 2Department of Nutritional Sciences, University of Wisconsin-Madison, Madison, Wisconsin; 3Department of Pathology, University of Wisconsin-Madison, Madison, Wisconsin; and 4Department of Medical Physiology, The Panum Institute, University of Copenhagen, Copenhagen, Denmark

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Enteral nutrients potentiate glucagon-like peptide-2 action and reduce dependence on parenteral nutrition in a rat model of human intestinal failure. Am J Physiol Gastrointest Liver Physiol 303: G610–G622, 2012. First published June 28, 2012; doi:10.1152/ajpgi.00184.2012.—Glucagon-like peptide-2 (GLP-2) is a nutrient-dependent, proglucagon-derived gut hormone that shows promise for the treatment of short bowel syndrome (SBS). Our objective was to investigate how combination GLP-2 + enteral nutrients (EN) affects intestinal adaption in a rat model that mimics severe human SBS and requires parenteral nutrition (PN). Male Sprague-Dawley rats were assigned to one of five groups and maintained with PN for 18 days: total parenteral nutrition (TPN) alone, TPN + GLP-2 (100 μg·kg−1·day−1), PN + EN + GLP-2 (TPN), EN + PN + EN + GLP-2 (18), and a nonsurgical oral reference group. Animals underwent massive distal bowel resection followed by jejunocolic anastomosis and placement of jugular catheters. Starting on postoperative day 4, rats in the EN groups were allowed ad libitum access to EN. Groups provided PN + EN + GLP-2 had their rate of PN reduced by 0.25 ml/day starting on postoperative day 6. Groups provided EN + PN + GLP-2 demonstrated significantly greater body weight gain with similar energy intake and a safe 80% reduction in PN compared with TPN + GLP-2. Groups provided PN + EN + GLP-2 for 7 or 18 days showed similar body weight gain, residual jejunal length, and digestive capacity. Groups provided PN + EN + GLP-2 showed increased jejunal GLP-2 receptor (GLP-2R), insulin-like growth factor-I (IGF-I), and IGF-binding protein-5 (IGFBP-5) expression. Treatment with TPN + GLP-2 demonstrated increased jejunal expression of epidermal growth factor. Cessation of GLP-2 after 7 days with continued EN sustained the majority of intestinal adaption and significantly increased expression of colonic proglucagon compared with PN + EN + GLP-2 for 18 days, and increased plasma GLP-2 concentrations compared with TPN alone. In summary, EN potentiate the intestinotrophic actions of GLP-2 by improving body weight gain allowing for a safe 80% reduction in PN with increased jejunal expression of GLP-2R, IGF-I, and IGFBP-5 following distal bowel resection in the rat.

GLUCAGON-LIKE PEPTIDE-2 (GLP-2) is a 33-amino acid, nutrient-dependent, proglucagon-derived gut hormone that is processed and secreted from the enteroendocrine L cells of the terminal ileum and colon in response to luminal nutrients, most notably carbohydrates and lipids (15). Following resection of the ileum, the colon is the primary site of GLP-2 production in both humans and rats (7, 28). Production of GLP-2 occurs in a pulse-like fashion ~30 min following ingestion of luminal nutrients and acts in both a paracrine and endocrine manner (49). Biologically active GLP-2 can be detected in serum readily after ingestion of luminal nutrients but is rapidly degraded (half-time ~7 min) by dipeptidylpeptidase-IV (DPP-IV) and is cleared in the urine (54). Previous studies have indicated the proliferative and anti-apoptotic effects of GLP-2 in intestinal mucosa for a variety of animal models (6, 11, 14). GLP-2 functions to maintain the integrity of the intestinal epithelium through enhanced mesenteric blood flow, resulting in increased digestion and absorption of luminal nutrients from an enhanced absorptive surface area (6, 11, 18, 19, 30, 53). The mechanism by which GLP-2 functions still remains unknown, but promising work has demonstrated multiple downstream mediators of action, including insulin-like growth factor-I (IGF-I), epidermal growth factor (EGF), vascular endothelial growth factor, keratinocyte growth factor, nitric oxide, vaso-active intestinal peptide, and ErbB ligands (12, 15, 18, 19, 27, 30, 35, 36, 49, 50). Phase III clinical trials have recently demonstrated that, in a randomized placebo-controlled trial, patients given teduglutide, a DPP-IV-resistant GLP-2 analog, had a statistically significant decrease in parenteral nutrition (PN) volume and in some cases were liberated from PN (21). In the near future, a GLP-2 analog will likely become available as an adjunct therapy for patients who suffer from intestinal failure (4, 9, 21, 24, 41).

Short bowel syndrome (SBS), a form of intestinal failure, encompasses a spectrum of disorders that occur when insufficient absorptive area of the small bowel results in malnutrition, weight loss, electrolyte abnormalities, severe diarrhea, and failure to thrive (3, 58). SBS affects ~30,000 patients in the United States yearly and requires PN for survival. In the pediatric population, the most common etiologies include necrotizing enterocolitis, malrotation with midgut volvulus and gastrochisis, compared with the adult population where segmental resection from inflammatory bowel disease (Crohn’s disease), malignancy, or mesenteric vascular accidents account for the majority of cases (3, 5, 56, 59). Management of SBS has evolved dramatically over the past two decades with improved PN formulations and support services, initiation of enteral nutrition (EN), surgical lengthening procedures, and intestinal transplantation (26, 56). Although PN is required for nutritional support and survival in patients with SBS, PN is associated with high morbidity, including recurrent episodes of catheter-related blood stream infections, sepsis, central venous

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thrombosis, and parenteral nutrition-associated liver disease (48, 55).

Attempts for exclusive EN in patients with severe SBS are often met with high morbidity and mortality secondary to large fluid losses, electrolyte abnormalities, malabsorption leading to malnutrition, poor wound healing, and failure to thrive. Several recent studies have highlighted the importance of trophic feeding to promote intestinal adaptation as mediated by intraluminal nutrients, gastrointestinal secretions, growth factors, and byproducts of digestions such as short-chain fatty acids (26, 33, 40, 46, 56). While GLP-2 has been met clinically with great success, a recent study indicating increased colonic dysplasia in a murine model of azoxymethane-induced colon cancer has raised the concern for GLP-2 as a cancer promoter (57). Few studies have investigated how administration of GLP-2 in combination with EN can result in intestinal adaptation (11, 26, 37–39). Given evidence that cessation of GLP-2 infusion reverses adaptive growth in humans with SBS (23) and in the current rat model (28), we assessed whether EN could sustain intestinal adaption after discontinuation of GLP-2. The objective was to investigate how following distal bowel resection, combination treatment with GLP-2 + EN affects intestinal adaption in a rat model that mimics severe human SBS and requires PN for survival.

MATERIALS AND METHODS

Animals and Experimental Design

The animal facilities and research protocols were approved by the University of Wisconsin Madison Institutional Animal Care and Use Committee. Male, 150- to 175-g Sprague-Dawley rats (Harlan, Indianapolis, IN) were housed in individual stainless steel cages with unlimited access to water in a room maintained at 22°C on a 12:12-h light-dark cycle. Animals were adjusted to the facility and fed a semipurified diet ad libitum. Three days before surgery, animals were transitioned to a liquid semielemental, low-residue diet that provided 1 kcal/ml with 12% energy from protein, 35% energy from fat, and 53% from carbohydrates (Abbott Laboratories, Columbus, OH).

On the day of surgery, rats were randomized to one of five treatment groups: total parenteral nutrition alone [TPN; n = 8(14)], TPN + GLP-2 [TPN + GLP-2; n = 5(8)], parenteral nutrition + GLP-2 for 7 days + EN [PN + EN + GLP-2 for 7 days; n = 7(10)], parenteral nutrition + GLP-2 for 18 days + EN [PN + EN + GLP-2 for 18 days; n = 5(8)], and an oral nonsurgical reference group [oral; n = 5(5)] (Fig. 1A). The number of animals assigned to each treatment group is shown in parentheses, and the number outside the parentheses indicates the final sample size at the conclusion of the 18-day experiment. A group provided exclusive EN as well as a group provided PN + EN without GLP-2 following resection were not included in our study due to prior experience, indicating that EN are not sufficient to promote intestinal adaptation and induce nearly 100% mortality in this distal bowel resection model of intestinal failure (31, 39, 51). Rats were maintained with either TPN or PN + EN for 18 days. The two main treatment effects were ± GLP-2 and ± EN. The dose of GLP-2 (100 μg·kg⁻¹·day⁻¹) is a physiological dose previously shown to assist with intestinal adaptation (39). The EN used consisted of a liquid, semielemental, nutritionally sufficient diet formulated for a rodent (TD.07244; Harland Teklad, Madison, WI) and provided 1 kcal/ml with 16.8% of energy from protein, 9.9% from fat, and 73.3% from carbohydrates (38).

Twelve hours before surgery, animals were fasted but allowed ad lib rum access to water. On the day of surgery, animals were anes-

![Experimental design demonstrating treatment groups](http://ajpgi.physiology.org/)

**Fig. 1.** Experimental design demonstrating treatment groups; total parenteral nutrition (TPN, n = 8), TPN + glucagon-like peptide-2 (TPN + GLP-2, n = 5), parenteral nutrition (PN) + enteral nutrition (EN) + GLP-2 for 7 days [PN + EN + GLP-2(7d), n = 7], PN + EN + GLP-2 for 18 days [PN + EN + GLP-2(18d), n = 5], and nutrition provided during the 18-day experiment. Nos. denote postoperative day (A). Resected animals underwent massive distal bowel resection (60% jejunooileal resection + ceccectomy and primary end-to-end jejunooileocolonic anastomosis) followed by placement of a 3-Fr central venous catheter in the right internal jugular vein. Forty centimeters distal to the ligament of Treitz to 1 cm distal to the cecum was resected (B).
followed by a 60% jejunoileal resection
in a 60% jejunoileal resection + cecectomy with a primary end-to-end jejunojejunal anastomosis (Fig. 1B). This resection removes the terminal ileum and cecum, which are the primary site of L cells and proglucagon/GLP-2 production (7, 28). The entire small bowel was eviscerated, and the ligament of Treitz was identified. Forty centimeters distal to the ligament of Treitz to 1 cm distal to the cecum was removed, followed by restoration of bowel continuity. A single surgeon, using a suture of 40 cm, measured the bowel that would remain. Following resection, 5 ml of warm 0.9% sodium chloride was instilled in the peritoneal cavity for resuscitation before the peritoneum was closed with absorbable suture, and the skin incision was closed with wound clips. Next, rats underwent cannulation of the superior vena cava with placement of a 3-Fr catheter to allow infusion of PN as previously described (28, 29, 37–39). Following surgery, all rats were recovered in individual wire-bottom cages and received 0.18 mg/kg intraperitoneal oxytetracycline hydrochloride (SAGENT Pharmaceuticals) every 6 h × 48 h for analgesia and 200 mg/kg of ampicillin every 12 h × 48 h for perioperative prophylaxis.

The isonitrogenous and isoeenergetic TPN was prepared aseptically as a total nutrient admixture using commercial preparation of amino acids, 20% dextrose, lipid emulsion, vitamins, nutrients, trace elements, and choline to meet the requirements of a rat. TPN was infused via a Harvard syringe pump (Harvard Apparatus, Holliston, MA) at 1 ml/h the day of surgery (day 0), advanced to 1.67 ml/h on postoperative day (POD) 1 (POD1), and finally advanced to a nutritionally sufficient rate of 2.5 ml/h by POD 2. Rats receiving TPN alone or TPN + GLP-2 received the following daily intake of nutrients on days 2–18: 64 kcal (238 kJ) (250 kcal or 1,046 kJ/kg body wt), 2.6 g protein (16.5% total energy), 1.7 g fat (24% total energy), and 11 g carbohydrates (59% total energy).

The two groups receiving EN were offered ad libitum access to EN starting on POD 4 in graduated feeding tubes. The same two groups received 2.5 ml/h of PN on POD 2–6 and then had the infusion rate reduced in stepwise fashion by 0.25 ml/day (10% decrease) until a final rate of 0.5 ml/h (80% decrease) was achieved by POD 14. Rats had their PN infusion rate maintained at 0.5 ml/h until the conclusion of the experiment on POD 18. With the stepwise reduction in PN infusion rate starting on POD 6, energy intake from EN slowly increased and provided ~80% of daily total energy intake by the conclusion of the study.

Groups treated with GLP-2 received 100 μg·kg−1·day−1 of human GLP-2 (33 amino acids, preproglucagon 126–158; CA Peptide Research, Napa, CA), which was reconstituted in sterile PBS and added daily to the PN. Vehicle (PBS) was infused in groups not given GLP-2. Daily body weight, volume of PN infused, volume of EN consumed, and urine output were recorded for all animals. After 18 days, animals were weighed, anesthetized with isofluorane, and killed by cardiac puncture and exsanguination within 10–20 min of disconnection from the continuous PN. The remaining small bowel and colon were removed for analysis.

Intestinal Composition, Histology, Enzyme Analysis

The bowel was sectioned into the duodenum, remaining jejunum, and colon, and the length of each section was determined by hanging the segment from a fixed point with a constant mass for a fixed amount of time. The jejunoileal anastomosis was identified, and 1 cm on either side was transected and discarded. The bowel was then flushed with ice-cold saline, weighed, and sectioned with more detailed analyses reserved for the jejunum and colon where significant adaptation occurs. The jejunum was sectioned on a chilled glass plate. The first 2 cm (0–2 cm) distal to the ligament of Treitz were used for measuring wet and dry intestinal mass, and the following 4 cm (2–6 cm) were used for RNA extraction and real-time quantitative PCR. The next segment (6–10 cm) was used to measure wet and dry mass of the mucosa only. The mucosa was separated from the muscularis propria and serosa by having a single surgeon incise the segment of intestine longitudinally and carefully scrape the mucosa with a glass slide as previously reported (43). The first 1 cm (10–11 cm) was fixed in 10% formalin, paraffin embedded, cut into 5-μm sections, and stained with hematoxylin and eosin (H&E) for histomorphology as previously described (28). Mucosal scraping from the next 4 cm (11–15 cm) was used to determine DNA concentration (32), protein concentration (bicinchoninic acid protein assay; Pierce Chemicals, Rockford, IL), sucrase activity (10), and alkaline phosphatase activity (alkaline phosphatase assay kit; BioVision, San Francisco, CA).

The colon was sectioned in a similar manner with the first 2 cm of intact tissue (0–2 cm) utilized for wet and dry intestinal mass and the following 1 cm (2–3 cm) for histomorphology. The next 2 cm (3–5 cm) of intact tissue were used to determine DNA concentration, protein concentration, sucrase activity, and alkaline phosphatase activity. The following 2 cm (5–7 cm) were then used for RNA extraction and quantitative PCR.

Jejunal Histomorphology, Apoptosis, Mitosis

Villus height, crypt depth. With the use of conventional light microscopy and H&E sections, villus height and crypt depth were analyzed. A single, experienced, blinded researcher obtained ×100 microscope pictures for the jejunum of all animals. With the use of well-oriented columns (i.e., one side of a crypt or villus in a longitudinal cross section), and a standard microscope ruler, 15 villi and 15 crypts were measured for each animal.

Apoptotic and Mitotic Index

Apoptosis and mitosis of the residual jejunum were determined using H&E-stained slides with the assistance of a pathologist (Hitt) who was blinded to the groups. Apoptosis, as determined from condensed chromatin, nuclear fragmentation, and formation of apoptotic bodies and mitosis as determined by spindle formation, chromatid condensation, and cytoskeleton rearrangement were assessed for each animal. Fifteen well-oriented crypt-villus axes were assessed for each animal, and the total number of cells for each side of a villus and crypt, as well as the number...
of apoptotic and mitotic cells for each villus and crypt, were assessed. Data are presented as an index, which is defined as the total number of mitotic or apoptotic cells divided by the total number of cells for each villus and crypt as previously described (28).

**Plasma Biochemical Analysis**

On the day of death, blood was collected by cardiac puncture into chilled syringes containing a final concentration of 1 mg/ml EDTA, 0.1 mM Dipoprotein A (MP Biomedicals, Aurora, OH), and 0.01 nM aprotinin (Calbiochem, La Jolla, CA). Plasma was isolated within 1 h of collection by centrifugation at 1,800 g for 15 min at 4°C. Plasma GLP-2 concentration was determined using a radioimmunoassay (RIA) with an antibody specific to the NH₂-terminus of the intact GLP-2 (20). Plasma IGF-I was measured by RIA after separation of IGF-binding proteins under acidic condition by HPLC with recovery of 85–90%, as previously reported (43–45).

**Biomolecular Analysis**

RNA, real-time PCR. Total RNA was extracted from the residual jejunum and colon using the TRIzol reagent (GIBCO-BRL Life

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**Table 1. Total daily energy intake from parenteral and enteral nutrition**

<table>
<thead>
<tr>
<th></th>
<th>TPN</th>
<th>TPN + GLP-2</th>
<th>PN + EN + GLP-2 (7 days)</th>
<th>PN + EN + GLP-2 (18 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>POD 2–4</td>
<td>64 (268)</td>
<td>64 (268)</td>
<td>64 (268)</td>
<td>64 (269)</td>
</tr>
<tr>
<td>POD 4–7</td>
<td>64 (268)</td>
<td>64 (268)</td>
<td>8 ± 3 (33 ± 11)</td>
<td>5 ± 4 (21 ± 17)</td>
</tr>
<tr>
<td>PN</td>
<td>64 (268)</td>
<td>64 (268)</td>
<td>24 ± 6 (100 ± 25)</td>
<td>27 ± 2 (113 ± 8)</td>
</tr>
<tr>
<td>EN</td>
<td>0</td>
<td>0</td>
<td>42 ± 3 (176 ± 13)</td>
<td>35 ± 4 (146 ± 17)</td>
</tr>
</tbody>
</table>

Values are mean ± SE energy intake/day expressed in kcal and kJ (in parentheses) from total parenteral nutrition (TPN), parenteral nutrition (PN), and enteral nutrition (EN) from postoperative days (POD) 2–18 following distal bowel resection (60% jejunoileal resection + cecectomy) and 18 days of PN. Total energy intake did not vary significantly across the groups for the 18-day study. GLP-2, glucagon-like peptide-2.

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**Fig. 3.** Residual jejunal length (A), jejunal mucosa dry mass (B), protein concentration (C), and DNA concentration (D) for all groups following distal bowel resection (60% jejunoileal resection + cecectomy) and 18 days of PN. Values are means ± SE. Means with different superscripts are statistically significant (P < 0.05).
Technologies, Grand Island, NY). Tissue (80–100 mg) was sectioned, total RNA extracts were quantified by absorbance at 260 nm, and integrity was checked using a 1.25% agarose/2.2 M formaldehyde gel electrophoresis staining for ribosomal bands using ethidium bromide. Expression of proglucagon, GLP-2 receptor (GLP-2R), and IGFI mRNA in the residual jejunum and colon was measured in a two-step reverse transcriptase real-time PCR (RT-qPCR) using SYBR green detection method as previously described (29, 47). β-Actin mRNA expression was determined for both segments and acted as a reference gene based on evidence that mean β-actin mRNA expression did not differ between treatment groups. Sequences for forward and reverse primers (Integrated DNA Technologies, Coralville, IA) were reported previously (28–30, 37, 38). Expression of EGF and insulin-like growth factor-binding protein-5 (IGFBP-5) mRNA was measured in the residual jejunum using TaqMan Gene Expression assay (Applied Biosystems, Foster City, CA) using the following rat primer kits: EGF (Rn00563336_m1) and IGFBP-5 (Rn00563116_m1). Expression of β-actin (Rn00667869_m1) mRNA was determined and acted as a reference gene based on evidence that mean β-actin mRNA expression did not differ between treatment groups. Data were analyzed using 7000 system software (Applied Biosystems).

**Statistical Analysis**

Treatment groups were analyzed using general linear models; differences among treatment groups were assessed with one-way ANOVA followed by the protected least-significant differences technique (SAS version 8.2; SAS Institute, Cary, NC). Statistics were performed on transformed data for results showing unequal variance among groups, and all data are presented as means ± SE; P < 0.05 was considered statistically significant.

**RESULTS**

**Body weight and energy intake.** Changes in mean body weight as a function of time are illustrated in Fig. 2. All groups had similar mean body weights (180–191 g) on the day of surgery as represented by day 0. All groups lost weight during the initial 48 h following surgical resection and regained their preoperative weight by POD 2–4. In the two groups given EN starting on POD 4, an immediate increase in mean body weight was observed, initially attributed to the mass of the EN consumed compared with groups provided TPN or TPN + GLP-2.

In the two groups provided PN + EN + GLP-2, there was an increase in mean body weight even with the stepwise reduction of PN rate, which started on POD 6 compared with groups provided TPN or TPN + GLP-2. The group provided PN + EN + GLP-2 for 7 days demonstrated a similar growth curve compared with the group provided PN + EN + GLP-2 for 18 days and had a mean 18-day body weight gain that was not significantly different between the two groups (59 ± 6 vs. 50 ± 3 g). Groups provided PN + EN, irrespective of duration of GLP-2 administration, had an 18-day body weight gain that was significantly greater compared with animals provided TPN or TPN + GLP-2 (50 vs. 29 g/18 days, P < 0.05). Total energy intake was 10–15% greater in groups provided PN + EN + GLP-2 on days 4–7 as opposed to TPN alone (71 vs. 64 kcal/day); however, energy intake between all groups was not statistically different from POD 8 to 18 (Table 1). There was not a significant difference in daily energy intake between groups provided EN.

**Intestinal adaptive growth.** Groups provided GLP-2 demonstrated increased residual jejunal length, as measured from the ligament of Treitz to the jejunocolic anastomosis, compared with the group provided TPN alone (Fig. 3A). In the group provided TPN alone, the residual jejunal length was noted to be 33.8 ± 1.6 cm compared with the 40 cm that remained following resection. There was no difference in residual jejunal length in groups provided PN + EN + GLP-2 for 7 or 18 days (43.4 ± 1.4 vs. 40.8 ± 1.7 cm). In the three groups provided GLP-2, the residual jejunum demonstrated significantly increased mucosal dry mass and concentrations of protein and DNA compared with the group provided TPN alone (Fig. 3, B–D). The group provided PN + EN + GLP-2 for 18 days
demonstrated a significant 36% increase in mucosal dry mass compared with the group treated with TPN + GLP-2 and a 30% increase compared with the group treated with PN + EN + GLP-2 for 7 days. There were no statistically significant differences with respect to protein concentration among groups given GLP-2; however, the group provided PN + EN + GLP-2 for 18 days had a statistically significant increase in DNA concentration compared with the group provided PN + EN + GLP-2 for 7 days.

Histology and mucosal enterocyte kinetics. Representative H&E-stained residual jejunal segments for the treatment groups are shown in Fig. 4. A significant increase in residual jejunal villus height was observed in the three groups treated with GLP-2 compared with the group provided TPN alone (Fig. 5A). There was no significant difference in villus height among the group provided TPN + GLP-2 compared with the group provided PN + EN + GLP-2 for 18 days; however, the group provided PN + EN + GLP-2 for 7 days demonstrated a 13% decrease in villus height compared with the group provided PN + EN + GLP-2 for 18 days and was not significantly different from the group provided TPN + GLP-2.

Consistent with villus height and mucosal DNA concentrations, residual jejunal mucosa cellularity was greatest in the group provided PN + EN + GLP-2 for 18 days (Fig. 5B). There was a significant 35% decrease in villus cellularity in the group provided PN + EN + GLP-2 for 7 days compared with the group provided PN + EN + GLP-2 for 18 days. The group provided TPN + GLP-2 demonstrated a significant 24% increase in residual jejunal villus cellularity compared with the TPN alone group but remained significantly less than the group treated with PN + EN + GLP-2 for 18 days.

Within the crypt compartment, treatment with GLP-2 resulted in a significant increase in crypt depth among groups compared with the group treated with TPN alone (Fig. 5C). Cellularity within the crypt compartment of the residual jejunum was greater in both groups treated with GLP-2 for 18 days. This increased crypt cellularity did not persist in the group treated with PN + EN + GLP-2 for 7 days and was not significantly different from the group treated with TPN alone (Fig. 5D). With respect to crypt mitotic index, as a marker for enterocyte proliferation, there was a general trend among groups treated with GLP-2 for an increased mitotic index compared with the group treated with TPN alone. However, only the group treated with PN + EN + GLP-2 for 7 days demonstrated a significant increase in crypt mitotic index compared with the group treated with TPN (5.7 vs. 2.2%, P <

Fig. 5. Mean residual jejunal villus height (A), villus cellularity (B), crypt depth (C), and crypt cellularity (D) for 15 well-orientated crypt/villus axes for all treatment groups following distal bowel resection (60% jejunooileal resection + cecectomy) and 18 days of PN. Values are means ± SE. Means with different superscripts are statistically significant (P < 0.05).
Crypt apoptotic index was analyzed and was not statistically different among resected groups as previously noted (42) (data not shown).

Digestive capacity. Sucrase activity as a marker of carbohydrate metabolism was markedly increased in those groups provided GLP-2 compared with the group provided TPN alone. The greatest increase in sucrase activity was observed in the group provided PN/GLP-2 for 18 days, which was increased significantly by 56% compared with the group provided TPN/GLP-2. There was no significant difference in sucrase activity in those groups given PN/GLP-2 for 7 or 18 days (Fig. 7A).

Plasma GLP-2 and IGF-I. In all groups that underwent resection and were treated with GLP-2, significantly elevated plasma GLP-2 concentrations were observed compared with the group treated with TPN alone. There was no difference among the groups treated with GLP-2 for 18 days. However, as expected, a noticeable 63% decrease in plasma GLP-2 concentration was observed in the group treated with PN + EN + GLP-2 for 7 days compared with the group treated with PN + EN + GLP-2 for 18 days. In addition, the group provided PN + EN + GLP-2 for 7 days had a significant 28% elevation in plasma GLP-2 concentration compared with the group treated with TPN alone and was greater than the usual range of 25–30 pmol/l observed in the nonsurgical oral reference group from our studies (29, 38, 39) (Fig. 8A). All groups that underwent resection had decreased plasma IGF-I concentrations compared with a nonsurgical oral reference group with a trend toward higher plasma IGF-I concentrations in the group treated with PN + EN + GLP-2 for 18 days ($P = 0.0575$) (Fig. 8B).

Proglucagon, GLP-2R, IGF-I, EGF, and IGFBP-5 expression. Following distal bowel resection, all groups demonstrated a significant 50–80% decrease in residual jejunal proglucagon expression compared with a nonsurgical oral reference group. In the groups provided GLP-2, there was a significant decrease in jejunal proglucagon expression compared with the group treated with TPN alone with a trend toward increased proglucagon expression when EN was provided (Fig. 9A).

Jejunal GLP-2R expression was 60% lower in the group provided TPN + GLP-2 compared with the TPN alone group; this decrease in GLP-2R was mitigated or reversed by EN in the groups provided PN + EN + GLP-2 for 7 or 18 days (Fig. 9B). In addition, residual jejunal IGF-I expression was increased significantly in the group provided PN + EN + GLP-2 for 18 days and the group maintained with TPN alone compared with TPN + GLP-2, whereas the group provided PN + EN + GLP-2 for 7 days demonstrated a 58% increase compared with TPN + GLP-2 (Fig. 9C).

Jejunal expression of IGFBP-5 was measured to explore the relationship between jejunal growth and expression of IGF-I given its modulation of IGF-I bioactivity (43). Treatment with TPN alone reduced IGFBP-5 expression to nearly undetectable levels compared with the oral reference group (Fig. 9D). Treatment with TPN + GLP-2 resulted in significantly in-
creased IGFBP-5 expression with a trend toward increased expression following the addition of EN.

Following resection, the group maintained with TPN alone demonstrated a 50% decline in EGF expression compared with the oral reference group. TPN + GLP-2 without EN resulted in a significant 150% increase in jejunal EGF expression compared with the group maintained with TPN alone. There was a trend toward decreased expression of EGF following the addition of EN, and the group maintained with PN + EN + GLP-2 for 18 days showed a significant decrease in EGF expression comparable to the group treated with TPN + GLP-2 (Fig. 9E).

Following massive distal bowel resection, the colon is the primary source of GLP-2, and the group treated with TPN alone demonstrated a 115% greater expression of colon proglucagon expression compared with a nonsurgical oral reference group. The group treated with PN + EN + GLP-2 for 18 days had a significant 73% decrease in colon proglucagon expression compared with the group treated with TPN alone. In contrast, the group treated with PN + EN + GLP-2 for 7 days showed a significant 65% increase in colon proglucagon expression compared with the group treated with PN + EN + GLP-2 for 18 days, and expression was similar to that observed in the group treated with TPN alone (Fig. 10A). In comparison, colon GLP-2R expression was increased significantly in those groups treated with PN + EN + GLP-2 compared with the nonsurgical orally fed group and the group treated with TPN alone. There was a significant 76% increase in colon GLP-2R expression in the group treated with PN + EN + GLP-2 for 18 days compared with the group treated with TPN + GLP-2; however, no difference was observed in the groups treated with PN + EN + GLP-2 for 7 or 18 days (Fig. 10B). With respect to IGF-I expression, groups treated with GLP-2 demonstrated a significant 56–62% increase in colon IGF-I expression compared with the group treated with TPN alone (Fig. 10C). Despite differences in proglucagon, IGF-I, and GLP-2R expression among the treatment groups, no significant difference with respect to colon length or mass was observed among the treatment groups (data not shown).

DISCUSSION

GLP-2 is an intestinotrophic proglucagon-derived gut hormone that is processed and secreted from the enteroendocrine cells of the terminal ileum and colon in response to luminal nutrients and following bowel resection. Humans that undergo massive bowel resection, including the ileum and proximal colon, similar to the massive distal bowel resection model used in the current study, have an impaired ability to synthesize and secrete GLP-2, resulting in limited intestinal adaptation and dependency on PN (22). Recent studies have indicated that human subjects given daily DPP-IV-resistant analogs of GLP-2 (teduglutide) have improved fluid and nutrient absorption and decreased dependency on PN (21). The superior role of enteral feeding in improving nutritional status as opposed to parenteral feeding and its associated complications have been well established (26, 52, 56, 58, 59). Moreover, luminal nutrients are a primary stimulus for intestinal adaptation, acting directly to provide nutrients to the proliferating enterocytes and indirectly to increase pancreaticobiliary secretions, intestinal blood flow, and trigger the release of enteroendocrine hormones, including GLP-2 (11). Enteral nutrients given in combination with GLP-2 induce synergistic intestinal adaptive growth as evidenced by increased mucosal cellularity and digestive capacity in rats with intestinal failure requiring PN for survival (37–39).

In this study, we have demonstrated that early EN can potentiate GLP-2 action and reduce dependency on PN using our well-established animal model of intestinal failure requiring PN for survival (17, 28, 29, 37, 39).

With respect to overall nutritional status as evidenced by gain in body weight, a clear superior advantage is seen in the two groups given PN + EN + GLP-2 compared with the group provided TPN alone or TPN + GLP-2. Both 18-day body weight gains and final body weight were statistically greater in the groups provided EN, whereas 18-day energy intake was not significantly different across groups. Provision of GLP-2 for 7 or 18 days in combination with PN + EN permitted a safe 80% stepwise reduction in PN and a decrease in associated morbidity, while still maintaining positive gains in body weight and superior final mean body mass compared with groups main-

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Fig. 8. Plasma bioactive GLP-2 (A) and insulin-like growth factor-I (IGF-I, B) concentrations as determined by RIA for all treatment groups following distal bowel resection (60% jejunoileal resection + cecectomy) and 18 days of PN and collected 10–20 min following discontinuation of PN infusion before death. Values are means ± SE. Means with different superscripts are statistically significant (P < 0.05).
tained with TPN alone or TPN + GLP-2. Taken together, we conclude that, following massive distal bowel resection resulting in severe SBS, combination PN + EN + GLP-2 is superior to TPN alone or TPN + GLP-2 and allows for greater gain in mean body weight while still allowing for a safe reduction in PN infusion rate and near complete liberation after 18 days.

The current model of intestinal failure, like the human condition of severe SBS, does not induce intestinal adaptive
growth because the primary sites for GLP-2 synthesis, the ileum and proximal colon, are resected (16, 17, 28, 29, 39). Accordingly, the plasma GLP-2 concentration of the resected group maintained with TPN alone was 26.3 pmol/l, a level below the sustained GLP-2 concentration of >50 pmol/l that is required for jejunal adaptation (29). As expected (28), the three groups treated with GLP-2 all showed significant increases in plasma GLP-2 concentration and corresponding increases in villus height, crypt depth, mucosal mass, and concentrations of protein and DNA consistent with increased mucosal cellularity relative to the TPN alone group. Moreover, the groups treated with GLP-2 with or without EN demonstrated increased residual jejunal length consistent with enhanced absorptive surface area. Given the anti-apoptotic effects of GLP-2 (49, 50), we speculate that the shorter than expected length of residual jejunum in the TPN alone group may reflect increased apoptosis of the muscularis propria. Furthermore, with respect to residual jejunal length, no significant difference was noted between the groups treated with EN + GLP-2 for 7 or 18 days, consistent with IGF-I expression in the muscularis layer (25).

Treatment with GLP-2 alone or in combination with PN + EN improved digestive function as evidenced by increased jejunal mucosal sucrase activity and alkaline phosphatase activity. As expected (28), the increase in sucrase activity was greater in those groups exposed to the sucrose substrate in the EN. The role of intestinal alkaline phosphatase has recently been shown to regulate intestinal lipid absorption, promote surfactant-like protein secretion, hydrolyze organic phosphates, and assist with detoxification of bacterial lipopolysaccharide (1, 8) and could explain the improved overall health and improved survival in those animals provided GLP-2 (28, 29). These results indicate that, following cessation of GLP-2 after 7 days with continued provision of EN, mucosal enterocytes undergo hypertrophy with sustained expression of digestive enzymes.

With the recent evidence indicating increased colonic dysplasia in a murine model of azoxymethane-induced colon cancer following exposure to GLP-2 (57), it would be prudent to limit the duration of GLP-2 exposure to the minimum necessary for intestinal adaptation. When comparing groups provided PN + EN + GLP-2 for either 7 or 18 days, overall 18-day body weight gain, longitudinal growth curves, final mean body weight, digestive capacity, and residual jejunal length were virtually identical between groups. This indicates that, with cessation of GLP-2 infusion after 7 days, the continued provision of EN sustains the majority of intestinal adaptation and crypt proliferation that is otherwise reversed in this model when EN were not provided and GLP-2 was stopped after 7 days (28).

Evidence suggests that IGFBP-5 can potentiate the intestinotropic action of IGF-I by increasing the availability of IGF-I to bind the IGF receptor (2, 17) and that IGF-I action is dependent on expression of IGFBP-5 (43). Interestingly, we observed that the increased jejunal and systemic growth noted in groups provided PN + EN + GLP-2 was associated with increased jejunal expression of both IGF-I and IGFBP-5. In contrast, the group treated with TPN alone did not show intestinal adaptation or increased IGFBP-5 expression, despite greater expression of IGF-I. Thus, we conclude that the increased IGFBP-5 expression stabilized the locally produced IGF-I, whose expression was increased in groups provided EN, resulting in increased IGF-I bioactivity and intestinal adaptation. This is consistent with the notion that IGF-I + IGFBP-5 act together to mediate GLP-2 action (Fig. 11) (2, 14, 34, 35, 43, 47).

Fig. 10. Colon proglucagon (A), GLP-2R (B), and IGF-I (C) mRNA expression for all treatment groups following distal bowel resection (60% jejunoileal resection + cecectomy) and 18 days of PN. Results are expressed as fold increases compared with the oral nonsurgical reference group. Means with different superscripts are statistically significant (P < 0.05).
Provision of EN in concert with GLP-2 for 7 or 18 days attenuated the decreases in jejunal expression of GLP-2R and IGF-I induced by continuous intravenous GLP-2 administration (Fig. 9). Given that expression of IGF-I was decreased when GLP-2 was administered without EN, the greater expression of EGF may help to explain the observed jejunal growth induced by GLP-2 alone in the current SBS model. Taken together, the data support the role of multiple downstream mediators of GLP-2 action during different physiological states.

The colon plays a vital role with respect to fluid balance, absorption of short-chain fatty acids, and proglucagon synthesis following massive distal bowel resection (3–5, 9, 26, 33, 40, 46, 48, 52, 55, 56). Previous studies with the current model indicate that the colon shows increased mass and expression of proglucagon and the GLP-2R following resection; however, the GLP-2 produced during a 12-day experiment was insufficient to allow for jejunal adaptation (29). Our current study confirmed a significant increase in colon proglucagon expression following massive distal bowel resection without evidence of jejunal adaptation in the TPN alone group. Continuous parenteral infusion of GLP-2 for 18 days with or without EN elevated plasma GLP-2 concentrations fourfold compared with TPN alone and appeared to inhibit endogenous GLP-2 production based on decreased colon proglucagon expression. Of interest, cessation of GLP-2 after 7 days with the continuation of EN resulted in significantly increased colon proglucagon expression compared with the group maintained with PN + EN + GLP-2 for 18 days, indicating the release of inhibition from the exogenous GLP-2 and induction of endogenous colonic proglucagon expression. This anatomic difference of increased proglucagon expression was seen in the colon, the primary site of remaining L cells following resection and not observed in the residual jejenum with limited L cells present. This is further supported by significantly increased plasma GLP-2 concentrations relative to TPN alone despite cessation of GLP-2 infusion 11 days before measurement.

In conclusion, we have extended research using our unique PN-dependent rat model of human intestinal failure by demonstrating that EN in combination with intravenous GLP-2 for 7 or 18 days results in equivalent and improved residual jejunal length and digestive capacity while still allowing for a safe 80% reduction of PN with continued gains in body weight compared with treatment with TPN ± GLP-2. Furthermore, when given in combination with EN, cessation of GLP-2 after 7 days resulted in increased colonic proglucagon expression with concurrent increased plasma GLP-2 concentrations, sustained intestinal adaptation, and the potential benefit of decreased duration of exogenous GLP-2 exposure. Treatment with PN + EN + GLP-2 for 7 or 18 days appears to utilize IGF-I as a downstream mediator of GLP-2 action, based on increased jejunal expression of GLP-2R, IGF-I, and IGFBP-5. In contrast, the jejunal growth induced by GLP-2 treatment without EN was associated with increased expression of EGF. These findings are consistent with evidence of multiple downstream mediators of GLP-2 action (13, 15, 49, 50, 60). Our findings have clinical implications and provide a nutritional strategy to help patients with intestinal failure decrease their dependence on PN and its associated complications.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.
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REFERENCES


